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Preliminary

Isolation of Genomic DNA from Japanese Red Pine *(Pinus densiflora)*

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The pine genome is 10 times the size of the human genome[1]. During its evolution, it might have been prominently amplified and dispersed to form complex families[2]. Multigene families reported on pine species were the genes for retrotransposons, alcohol dehydrogenases, phenylalanine ammonia-lyases and stilbene synthases[2,3]. This is a main reason why high molecular weight (HMW) DNA is required to study pine genes. Here, we discussed how such high molecular weight DNA is extractable from Japanese red pine. DNA preparation were examined by conventional CTAB method and the method for HMW DNA preparation[4,5] with modification.

First, we have examined if HMW DNA over 100 kbp is extractable from pine trees. Briefly, the sample was ground to fine powder with liquid nitrogen. The nuclei were isolated from the powder and embedded into agarose to keep HMW during further handlings. The nuclei in agarose were digested with proteinase K. The digested DNA samples in agarose were analyzed by pulse field electrophoresis. The enzyme was completely inactivated with a proteinase inhibitor when they were used for PCR template or Southern analysis.

When whole seeds of the Japanese red pine were used for the DNA extraction, the yields were extremely low. Then the seed coats were removed by using a food processor but HMW DNA was recovered with low yield. After pulse field electrophoresis, the gel image showed smear bands from ca. 200 to 23 kbp with a strong band at the origin. The low yield may be ascribed to this strong band, or some DNA-contaminants complex, which made DNA extraction difficult. Thus sample source was changed from seeds to seedlings.

During the DNA extraction, pine seedlings tend to make DNA preparation red to brown. In order to avoid this problem, original alkaline washing buffer were neutralized, though it made difficult to remove acidic polysaccharides. The nuclei were embedded in agarose microbeads or plugs. The quality and size of the HMW DNA was evaluated by inverse field gel electrophoresis.

After the electrophoresis, the gel image showed smear bands with their DNA sizes from origin to 23 kbp. At the origin of the gel, no strong band was observed. The genomic DNA in the agarose plugs showed higher size distribution in the seedlings comparing with the case of seeds. The yields was roughly estimated on the plugs to be around 3.2 μg/g fresh wt. The prepared samples were successfully amplified stilbene synthase gene fragment but unable to be completely digested with some restriction enzymes. The low yield increases relative amounts of contaminant, which may inhibit enzyme activities. Isolation of nuclei may be a key step for the DNA extraction.

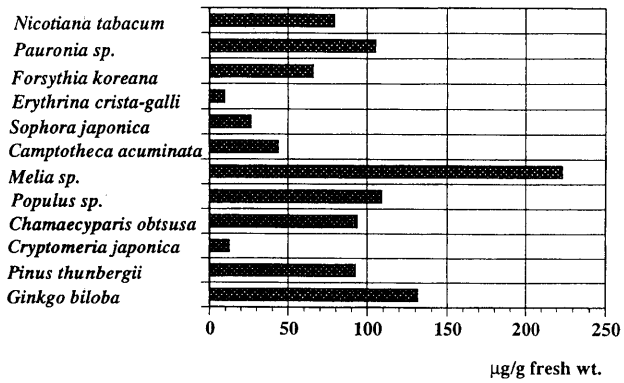

Fig. 1. Yields of genomic DNA from trees.

Genomic DNAs were extracted from 11 tree species by CTAB method (Figure 1). Most of the materials were young leaves or seedlings except tobacco and Ginkgo. The former was BY2 suspension cells and the latter was seeds, respectively. In the case of pines, yields were improved as high as 163 μg/g fresh weight if young hypocotyls and cotyledons without roots and seed coats were used. The quality of DNA was evaluated by spectra from 220 to 320 nm and by electrophoresis on 1 % agarose gel. The A260/A280 ratios of the prepared genomic DNAs were ca. 2.0, indicating the DNA with high quality. Most of the gel images indicated that the molecular size was over 23 kbp and less than 90 kbp without smear band. The DNA preparations showed good quality for constructing conventional genomic libraries.

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